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RESEARCH ARTICLE

Protective activities of Nigella sativa and its active gradient Thymoquinone on the carcinogenic effects of N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) on Wistar rats

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ABSTRACT

Alterations in some biochemical parameters were studied in male Wistar rats after a general assessment were carried out. Experimental groups were administered with 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) followed by 5% Sodium ascorbate (Na-As), then they were post treated with Nigella sativa (N. sativa) by an inter- gastric luminal gavage or with Thymoquinone (TQ) by an inter- peritoneal injection. Blood samples were collected after a 32 weeks' experiment. TQ seemed to have a powerful inhibitory effect on total cholesterol and triacylglycerol levels. While levels of A/G ratio, ALT and AST were highly decreased by both of N. sativa and TQ. These findings may rely the lipid modifying and hepato-protective effects of N. sativa crude oil and its main constituent TQ on mammals. Thus, further investigations are required.

Keywords: N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN), Sodium ascorbate (Na-As), Nigella sativa (N. sativa), Thymoquinone (TQ)

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INTRODUCTION

A total of 50 male Wistar rats were obtained at 6 weeks of age from the Helwan Breeding Facility of Helwan University, Cairo, Egypt. The rats were divided into 4 groups according to their body weight to minimize inter-group standard errors, then housed 4 or 5 per a plastic cage with wood chips for bedding. They were maintained at a temperature of 22+1℃, humidity of 55+5% and lighting of 12hr-12hr (light/dark) cycle. After four weeks of acclimatization the experiment was commenced to the animal house conditions. Pelleted diet and tap water were available ad libitium. Animal Care Facility of the Zoology Department, Faculty of Science, Tanta University, Egypt approved the experimental design.

MATERIALS AND METHODS

Chemicals

BBN was purchased from Sigma-Aldrich (Cairo, Egypt). While Sodium L-ascorbate (Na-As) was obtained from Al Gomhoria Co. (Tanta, Egypt). However, the crude extra virgin oil extracted from fresh N. sativa seeds was purchased from Al Serga press (Cairo, Egypt), it was refrigerated and protected from light exposure in dark glass bottles. Finally, TQ was obtained from Life Trade-Egypt (Cairo, Egypt).

Experimental design

Animals were observed daily to assess general health, clinical signs and mortality. Body weights, food and water consumptions were measured weekly. The treatment plan was explained by the experimental protocol in Figure 1. Carcinogenic BBN was given daily for 2 months to group 2, 3 and 4 in drinking water as 0.05% (1ml/2L). After BBN administration, the tumor promoter Na-As was given daily for 5 months till the end of experiment to group 2, 3 and 4 in drinking water as 5% (100gm/2L). Simultaneously with Na-As, N. sativa crude oil was given 5 days a week for 5 months to group 3 by intergastric (i.g.) injections as 0.05ml (200mg/kg b.wt.). TQ was dissolved in Alcohol and normal saline solution (0.024gm/7ml), then it was also administered with Na-As given 2 days a week for 5 months to group 4 intraperitoneally (i.p.) as 0.5ml (10mg/kg b.wt.).

Blood biochemical analysis

All animals were fastened overnight and killed under deep ether anaesthesia. Blood samples were collected from the abdominal aorta in a heparinized glass tube. Serum was separated by centrifugation at 3000 r.p.m. for 15 minutes, then stored at -20ºC. Lipid profile (including cholesterol, triglycerides, HDL and LDL) and liver functions (including total protein, A/G ratio, bilirubin, ALT and AST) tests were all performed using kits supplied by Sigma-Aldrich Company (Cairo, Egypt).

Statistical analysis

Mean and standard deviation values were calculated using Excel worksheets. While the significant difference of groups mean values were analyzed using a one-way ANOVA test, from the SPSS program version 19.0, where $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

General assessment

In the BBN-treated groups, two rats from group 2 and one rat from group 3 and 4 were moribund before the end point of the scheduled sacrifice. Therefore, their data were not included in the study. The growth rates in all BBN administered groups were significantly decreased if compared to the control group (Figure 2). However, this weight loss of BBN administered groups can be generally attributed to the obvious reduction in the water intake and food consumption as Table 1 demonstrates. Although Thymoquinone in group 4 had the lowest results of body weights among experimental groups, but still it managed to improve the food consumption near to the normal value found in the control group. In consistent with our results (Murai et al., 2005) has explained in his research that administrating BBN for two rats' strains (SD/cShi and SD/gShi) has resulted in decreasing the final average body weights approximately by 3-7%. In another study (Dollah et al., 2013) has discussed the effect of N. sativa at different doses on Sprague Dawley rats where it significantly reduced their average body weights. More recently, (Abduallah et al., 2017) has also noticed a high significant reduction in the mean body weights of female Wistar rats after their treatment with N. sativa extract (Thymoquinone) at different doses.

Organs weights

The absolute liver, kidneys and spleen weights results were all reduced by using N. sativa. On the other hand, the use of Thymoquinone only decreased the liver and spleen absolute weights, while the left and right kidneys results remained constant as illustrated in Table 2. In parallel with our findings (Salim et al., 2014) has recorded that Thymoquinone at different doses has managed to reduce the liver and spleen weights in the BALB/c mice. N. sativa administration seemed to have no powerful effect on the lipid profile parameters as it slightly decreased the total cholesterol and low-density lipoprotein (LDL) levels, whereas the triacylglycerol levels were highly increased. At the contrary Thymoquinone seemed to have a powerful effect on these parameters as it significantly decreased the total cholesterol levels as shown in Figure 3, triacylglycerol and low-density lipoprotein (LDL) levels were highly reduced as well. However, the high-density lipoprotein (HDL) levels were almost negligible for both treatments (Table 3).

In a previous study made by (El-Dakhakhny et al., 2000) it was reported that N. sativa has an anti-obesity property, the main reasons for this are not so clear, but factors such as reduction of appetite are proposed. In another similar study by (Asgary et al., 2015) it was found that different preparations of N. sativa including seed powder, seed oil, seed extract and Thymoquinone were found to reduce the levels of total cholesterol and triglycerides but the effect on HDL was not significant, this lipid modifying effect has made it a promising natural therapy for dyslipidemia after it have been reported to be safe and well tolerated. Another research has discussed the hypoglycemic properties of N. sativa fixed and essential oil as they have improved the lipid profile parameters including cholesterol, triglycerides and LDL after their administration for eight weeks was commenced by (Sultan et al., 2014) where he suggested their use in the treatment or prevention of diabetes and its related health conditions.

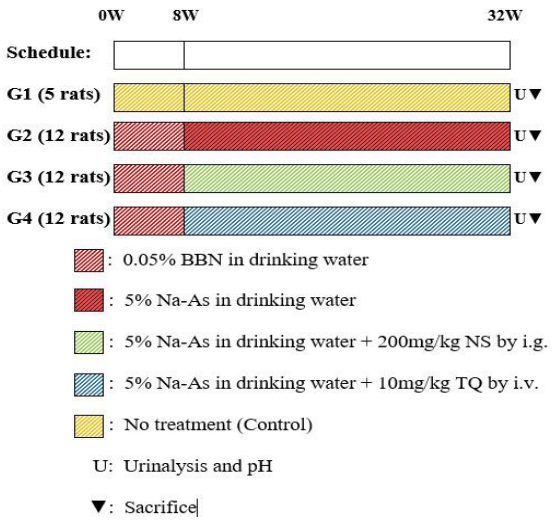

Figure 1. Experimental design.

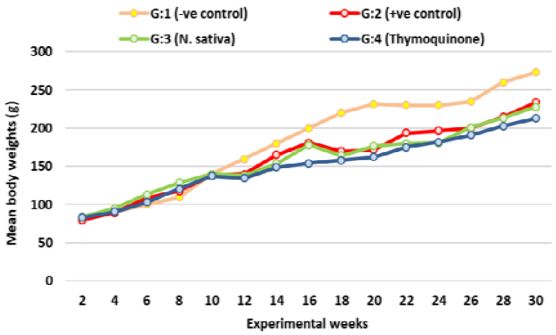

Figure 2. Growth curve.

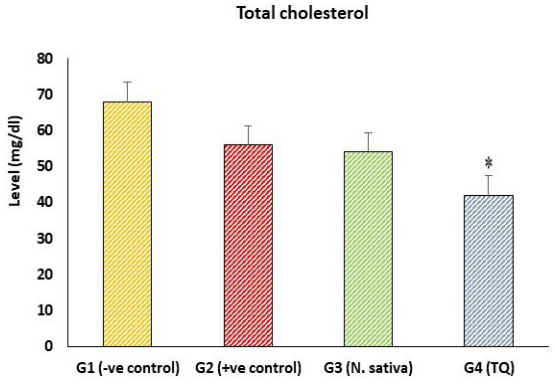

Figure 3. Levels of total cholesterol.

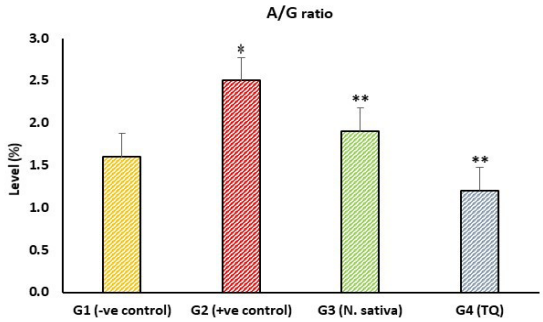

Figure 4. Levels of albumin to globulin ratio (A/G).

Nader et al., 2010 has also confirmed that thymoquinone caused a dramatic decrease in the total cholesterol, triglycerides and LDL levels, while the HDL level was increased through modulating the oxidative stress, this gave it a protective effect against the development of atherosclerosis in animals that received a high lipid diet in their regime.

Liver functions

The use of N. sativa showed a slight increase on the total protein and bilirubin levels, while it exerted a significant decrease on the albumin to globulin (A/G) ratio as seen in Figure 4, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were highly decreased as well. Thymoquinone treatment showed a similar effect of N. sativa but as a more powerful agent, this was illustrated in Table 4. In agreement with our output data (Dollah et al., 2013) and (Rahmani et al., 2014) had both reported that N. sativa has a hepato-protective effect as it shows no toxic effect on the liver tissues since it managed to reduce both the levels of ALT and AST. (Saricicека et al., 2014) seemed to have a similar result as he reported that administrating N. sativa, N. sativa oil and Thymoquinone to rats had caused a decrease in the A/G ratio, ALT and AST levels, generally the liver functions were - improved and the hepatic fibrosis values were significantly reduced in compare with the DMN-treated group. Furthermore, (Abdel- Moneim et al., 2013) demonstrated in his work how the use of N. sativa and Zingiber officinale separately or even combined had greatly improved the altered liver enzymes level including total bilirubin, albumin, ALT and AST in experimentally induced-HCV rats through their hepato-protective effect. (Linjawi et al., 2015) had also noticed that thymoquinone and black cumin seed oil were able to reduce the activity of AST along with other tumour markers, which ultimately provided a protective effect against breast cancer.

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Table 1. Water Intake and Food Consumption of Male Rats Treated with *Nigella sativa* or Thymoquinone after BBN Administration Followed by Sodium ascorbate

Group	No. of rats	Treatment	Water intake (g/rat/day)	Food consumption (g/rat/day)
1	12	Control	32±11[a]	34±15
2	10	BBN→Na-As	19±7	22±9
3	11	BBN→Na-As+*N. sativa*	18±9**	19±18
4	11	BBN→Na-As+TQ	17±8	32±10**

[a] Mean ± SD; BBN: N-butyl-N-(4-hydroxybutyl) nitrosamine; Na- As: sodium ascorbate; N. sativa: Nigella sativa; TQ: thymoquinone; ** vs. group 2 at P<0.05 (ANOVA test).

Table 2. Absolute and Relative Organs Weight of Male Rats Treated with *Nigella sativa* or Thymoquinone after BBN Administration Followed by Sodium ascorbate

Group	No. of rats	Treatment	Liver weight (g) (%)	Kidney weight (g) (%)		Spleen weight (g) (%)
				Right	Left	
1	12	Control	7.1±1.1[a] 2.6[b]	0.76±0.1 0.28	0.76±0.1 0.28	0.64±0.1 0.24
2	10	BBN →Na-As	7.8±1.1* 3.4	0.73±0.1 0.31	0.74±0.1 0.32	0.56±0.1 0.24
3	11	BBN →Na-As + N. sativa	7.5±0.7 3.3	0.71±0.1 0.32	0.70±0.1 0.31	0.53±0.1 0.23
4	11	BBN →Na-As + TQ	7.2±0.5 3.4	0.73±0.1 0.34	0.73±0.1 0.34	0.52±0.0 0.24

[a] Mean ± SD; [b] Relative organ weight (%); BBN: *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine; Na-As: sodium ascorbate; *N. sativa*: *Nigella sativa*; TQ: thymoquinone; * vs. group 1 at $P < 0.05$ (ANOVA test).

Table 3. Lipid Profile of Male Rats Treated with *Nigella sativa* or Thymoquinone after BBN Administration Followed by Sodium ascorbate

Group	No. of samples	Treatment	Triacylglycerol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	6	Control	53±0.0[a]	16±0.0	41±0.0
2	6	BBN →Na-As	74±57	13±2.8	28±5.6
3	6	BBN →Na-As + N. sativa	84±10	14±3.0	24±12
4	6	BBN →Na-As + TQ	58±25	9±0.1	25±0.1

[a] Mean ± SD; HDL: high density lipoprotein; LDL: low density lipoprotein; BBN: *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine; Na-As: sodium ascorbate; *N. sativa*: *Nigella sativa*; TQ: thymoquinone.

Table 4. Liver Functions of Male Rats Treated with *Nigella sativa* or Thymoquinone after BBN Administration Followed by Sodium ascorbate

Group	No. of samples	Treatment	Total protein (mg/dl)	Bilirubin (mg/dl)	ALT (mg/dl)	AST (mg/dl)
1	6	Control	5.7±0.0[a]	0.12±0.0	33±0.0	67±0.0
2	6	BBN→Na-As	5.3±0.1	0.08±0.1	71±7.7	128±45
3	6	BBN→Na-As + *N. sativa*	5.6±0.3	0.10±0.1	48±8.1	80±12
4	6	BBN→Na-As+ TQ	6.0±36	0.15±0.1	37±36	65±47

[a] Mean ± SD; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BBN: *N*-butyl-*N*-(4- hydroxybutyl) nitrosamine; Na-As: sodium ascorbate; *N. sativa*: *Nigella sativa*; TQ: thymoquinone.

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